Vallean

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1. Seed Transmission and Sterility Studies of Two Strains of Tobacco Ringspot.
2. A Virus Disease of Delphinium and Tobacco.
3. A Virus Disease of Plum and Peach.

BULLETIN NO. 327.
(RESEARCH BULLETIN)

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BULLETIN NO. 327.

(RESEARCH BULLETIN)

By W. D. VALLEAU

1. Seed Transmission and Sterility Studies of Two Strains of Tobacco Ringspot.

Ringspot is perhaps the most prevalent virus disease of tobacco in Kentucky, with the exception of tobacco mosaic. Sometimes ringspot causes nearly complete destruction of individual plants. Therefore it appears to be a potential menace to the tobacco industry. In addition to affecting tobacco, it causes a disease of cucumbers. It is perhaps a more common cause of cucumber mosaic, except in commercial vegetable and potato-growing areas, than the viruses which cause the so-called typical cucumber mosaic. It also causes a disease of potato which, however, does little damage. The ringspot virus is widely distributed in Kentucky. This raises the question as to the agencies of distribution and the reason for its wider distribution than certain other virus diseases of tobacco, some of which appear to be limited largely to vegetable-growing areas (11, 21). Tobacco mosaic is carried from one farm to another and probably from one region to another, in dried and manufactured tobacco (19, 20). Fortunately the other known virus diseases of tobacco, including ringspot, are not carried in this way.

Potatoes appear to play an important part in the distribution of viruses affecting other crop plants (21, 3). While ringspot sometimes affects potatoes, the evidence does not lead one to suppose that potatoes are the principal means of distribution of the tobacco ringspot virus. Other potato viruses, veinbanding, for example, are not so commonly found away from potato-growing areas as ringspot. For these and other reasons it has been suspected, since 1925, that the virus of tobacco ring-

spot is seed-borne. Experimental evidence supporting this view was not obtained by the writer until the fall of 1929.* This appears to be the first demonstration of the transmission of a virus thru tobacco seed. It is not at all certain that the wide distribution of ringspot in this and other areas can be attributed entirely to infected tobacco seed.

TWO STRAINS OF THE RINGSPOT VIRUS

In published descriptions (6) of ringspot of tobacco only one type has been recognized but there are at least two distinct strains of the virus. The commonly recognized symptoms of ringspot on leaves are large, chlorotic and necrotic ring-and-line patterns (figures 1, 3). Unaffected leaves retain about their normal color and appearance. Usually the color of the chlorotic patterns is various shades of green, with little if any yellowing. Another strain of the virus, however, causes the patterns to become distinctly yellow (figures 1, 9). Whole leaves of affected plants may bleach slightly (figure 2) or turn a light yellowish-green to nearly white. In suckers of white Burley tobacco following cutting, two distinct kinds of symptoms may develop, depending upon which strain of the virus is present. On some plants the suckers may be golden yellow with few or no necrotic rings. On others the suckers are green but have rings typical of ringspot. Inoculations from each type resulted in typical ringspot in Turkish tobacco. The symptoms on leaves of plants inoculated from yellow suckers usually were several days longer in developing than on plants inoculated from green plants having the typical rings. Seed transmission studies of the virus in green pattern and yellow pattern plants have demonstrated that there are two strains of the ringspot virus which will be designated green ringspot and yellow ringspot, respectively.

Affected seedlings from seed of green ringspot plants remain green and usually nearly normal in appearance thruout their lives, rarely developing ringspot patterns. Seedlings from yellow ringspot plants turn yellow soon after germination and

* 42nd Annual Report, Kentucky Agr. Expt. Sta. 1929, p. 17, and Ky. Agr. Expt. Sta. Bul. 309, plate 1, figure D.

have a bleached yellowish-green appearance thruout their growth. Seed transmission of the yellow ringspot virus is, therefore, readily demonstrated.

The two types of the ringspot disease usually can be distinguished readily in vigorously growing Turkish tobacco plants in the greenhouse. The early symptoms which follow inoculation may be identical. They are characterized by necrotic single, double or triple rings on the older inoculated leaves or single rings or necrotic spots on the younger, more tender, rubbed leaves. In yellow ringspot there is soon a marked tendency toward yellowing, especially in the new leaves. The yellowing may develop in small spots which may increase in size, or chlorotic yellowish rings may develop. The tissue in the vicinity soon yellows and develops into prominent spots (figure 9). In green ringspot the chlorotic line patterns may develop in the same way except that the lighter colored tissue is light green instead of yellow. Following the period of active development of necrotic rings, plants affected with the green type grow nearly normally, but the leaves of yellow ringspot plants gradually become chlorotic toward the tip or for some distance back from the tip. These leaves do not lose their chlorophyll completely but have a bleached light green or yellow to whitish appearance (figures 2, 9).

TWO TYPES OF RINGSPOT MAY AFFECT POTATOES

In previous publications (11, p. 387, and 21) the ringspot virus was reported in Cobbler potatoes and because of the yellow patterns on the leaves it was suggested that it might be identical with Aucuba mosaic as described by Quanjer (15). Thru the kindness of Dr. C. S. Schultz, Cobbler potatoes affected with Aucuba mosaic were obtained but ringspot was not transferred from them to tobacco, either by the usual rubbing method or by grafting. The Aucuba symptoms on Cobbler leaves were different from those produced by ringspot. Therefore it is concluded that the two diseases are distinct.

The green type of ringspot has now been obtained from 5 potato plants of different origin. It was obtained in three seed-

ling potato plants grown for one season on the Station farm; from a Cobbler potato plant, from which it was transferred three successive tuber generations; and from a potato plant (variety unknown) growing near a farmer's tobacco bed. From the Cobbler plant and the unknown variety both the healthy-potato

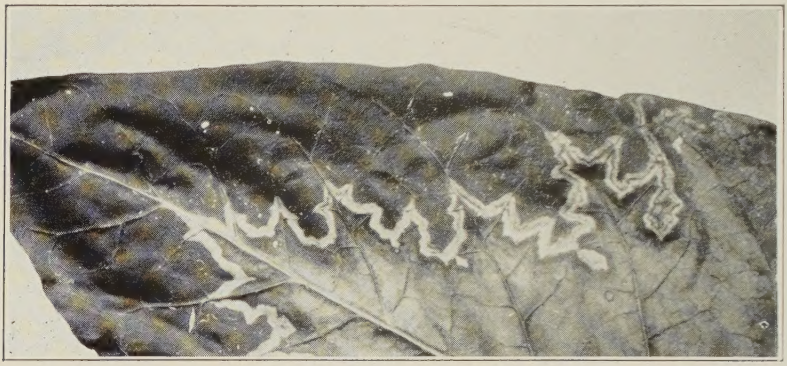

Figure 1. A portion of a leaf of Burley tobacco photographed July 28, 1922. The bleached appearance is typical of the yellow type of ringspot as it sometimes occurs in the field.

virus and ringspot were transferred. Repeated successful transfers from the Cobbler plant, and the yellowing of its young leaves, often in the form of partial rings, leave no doubt that the ringspot virus may become systemic in Cobbler. Only the tobacco ringspot virus was transferred to tobacco in the greenhouse from potato plants raised from field-grown tubers (seedlings). In each case the transfer from potato to tobacco was repeated successfully. Affected potato plants often show considerable yellowing of the foliage, especially on the first leaves of new plants, but at times symptoms may be absent.

Yellow ringspot has been transferred from tobacco to a seedling potato plant by grafting. It was then transferred again to tobacco from new growth of the potato by the rubbing method. In one instance a tobacco plant inoculated from a Cobbler potato plant showed evidence of the healthy-potato and veinbanding viruses and of an unknown virus. A transfer from the tobacco plant to cucumber resulted in a ringspot-like disease

and transfers from the cucumber to tobacco plants resulted in typical yellow ringspot. No consistent differences have been observed between the symptoms of yellow ringspot and green ringspot in potatoes. Henderson and Wingard (9) report successful transfer of ringspot to certain varieties of potato but the virus appeared to be purely local. Cobbler was not susceptible to this type of injury. The writer's tests with Cobbler always have given negative results by the rubbing method but positive by grafting.

OTHER HOSTS OF THE RINGSPOT VIRUS

Henderson and Wingard (9) report successful transfer of ringspot from a number of naturally infected annuals; namely, jimson, cantaloupe, possibly squash, petunia, and from naturally infected sweet clover, but were not certain that they obtained it from any perennials. They transferred a virus which they think may be ringspot from the perennial yellow ironweed, *Verbesina alternifolia* Britton. Fenne (5) claims to have demonstrated that this is a common carrier of the ringspot virus.

E. M. Johnson (11) reported successful transfer of ringspot from *Physalis* sp. and from horsenettle, *Solanum carolinense*. In a further study by the writer, of the viruses contained in solanaceous weeds, ringspot was transferred to tobacco from about 10 plants of horsenettle. Horsenettle appears to be a common carrier of this virus.

SEED TRANSMISSION OF THE GREEN RINGSPOT VIRUS

Since 1922, when the writer first recognized the infectious nature of tobacco ringspot,* records have been kept of the first appearances of the disease in the fields on the Experiment Station farm. In the first records of viruses, made about three weeks after setting tobacco, mosaic and ringspot always occurred

* The first description of ringspot of tobacco appears to have been made by the writer in 1921 (Plant Disease Survey Bulletin 5, p. 107, 1921), but no name was then given the disease. It was thought at that time to be different from mosaic but of the same general nature. In 1922 it was given the name, Hieroglyphics, and a report made of its successful transfer by mechanical means (Plant Disease Bulletin Supplement 26, p. 142, 1923) but as Fromme had referred to the same disease as ringspot (Plant Disease Survey Bul. 6, p. 83, 1922) this latter name was adopted.

and were usually the only viruses recorded. In 1930, for example, in a total of 57,350 Burley plants inspected, there were 94, or .16 percent, mosaic plants and 322 or .56 percent ringspot

Figure 2. On the left are two chlorotic leaves from near the top of a Turkish tobacco plant inoculated when young with yellow ringspot. On the right a leaf from a similar position on plant 7823-15-2 infected thru the seed with green ringspot.

plants. This early appearance of ringspot suggested the possibility of seed transmission. In inspecting plant beds in the vicinity of Lexington when the cotton was first removed, occasional plants were found affected with ringspot and one field was found, in 1925, in which 5.7 percent of the plants were affected with ringspot. A careful search was made thru this field for possible weed hosts but the field was very free from all kinds of weeds. The relatively even distribution of the disease suggested seed-bed infection and a study of the bed revealed a number of ringspot-affected plants scattered thru it. These observations could not be considered proof of seed transmission but they were suggestive.

In the fall of 1929 a flat was sown by Dr. E. M. Johnson with seed from a plant affected with the green type of ringspot.*

* The report given by Dr. Johnson of seed transmission of ringspot (11, page 302) is based on these results.

The plants were used by the writer during Dr. Johnson's leave of absence but without knowledge that the seed used was from a ringspot plant. While most of the plants were normal, a few, which were set in tumblers, made such poor growth and had such a peculiar appearance that they were set aside and not used for inoculation. Five others which appeared somewhat more normal and made better growth were used for inoculation but the fact of their peculiar appearance was recorded. The chlorotic symptoms on the edges of the leaves of these plants at the time of inoculation and subsequently were suggestive of a symptom of ringspot which often occurs in suckers of topped or harvested plants in the field, following a cool period. The plants grew slowly, the leaves were smaller than normal and rigid, and the edges of the distal portion of some of the leaves were somewhat chlorotic to necrotic and imperfectly developt, giving the leaf tip a slightly constricted appearance. These symptoms were present in all the plants in question. Inoculations from these plants, both the inoculated and the uninoculated, to healthy Turkish tobacco plants resulted in typical ringspot. One of the suspected uninoculated plants was transferred to a ½-gallon jar (No. 5925) and grown to maturity. During its growth no rings or any further symptoms of ringspot developt but transfers from it to healthy tobacco plants resulted in typical ringspot. Seed of this plant was saved for further study.

Early in 1930 another flat was sowed with seed from a green-ringspot Turkish tobacco plant. In this flat 2 plants developt necrotic patterns typical of ringspot (figure 3A). The plants were not exposed to low temperatures and consequently other affected plants may have escaped notice.

In the fall of 1930 seed was collected from typical ringspot-affected Burley and dark-tobacco plants in the field and from Turkish plants in the greenhouse. Seed was also collected from a dark-tobacco plant affected with ringspot which had two entire leaves bleached nearly white, seedlings of which will be discussed later. Seeds of 14 collections were sown in rows across a bench in the greenhouse September 17, 1930. When the largest plants were about 6 inches high they were pulled and

examined. All of the plants in the first 10 rows were pulled but no suspicious plants were noticed. From three rows only the largest plants were pulled and the smaller ones allowed to grow and were later pulled and carefully examined. Two Turkish plants with chlorosis along the edges of the leaves were found in one row, and one in another, these were transplanted. In about three weeks a few necrotic spots developt on the leaves of

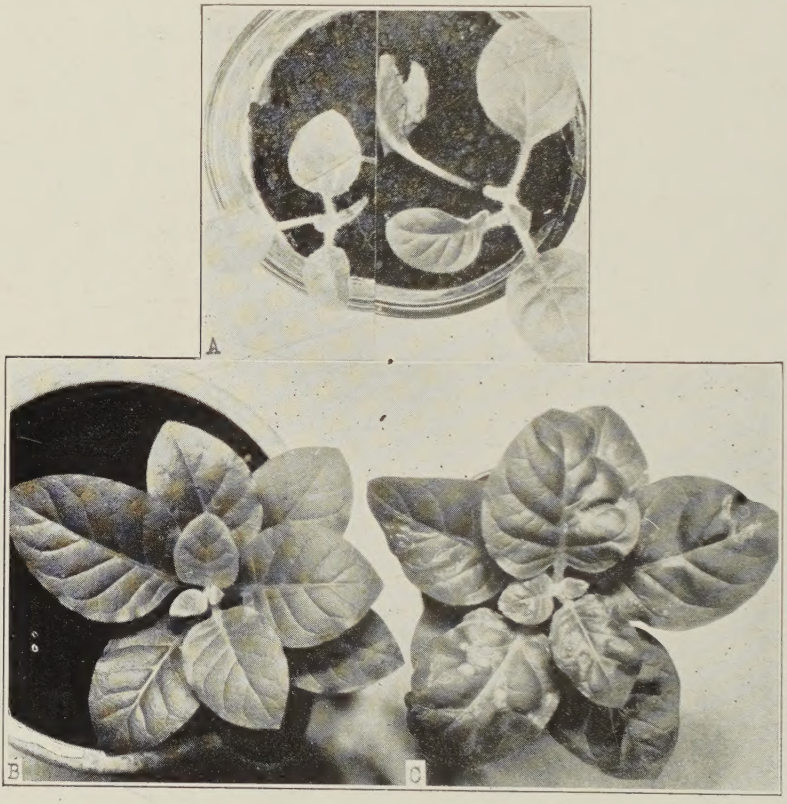

Figure 3. Seed transmission of green ringspot of Turkish tobacco. A.—In a flat of several hundred Turkish tobacco plants grown from seed of a ringspot plant these two were the only ones which developt ringspot patterns. Photographed March 29, 1921. B.—Turkish tobacco plant 7823-16-1, affected with the green ringspot virus thru the seed. The seed was from plant 5925 also affected with ringspot thru the seed. No ringspot symptoms are in evidence and except for slow growth, a rigid appearance of the leaves and rather short internodes the plant appears normal. C.—A Turkish tobacco plant inoculated January 10, 1931, with inoculum from B. Photographed January 21, 1931.

each plant. Inoculations to healthy plants resulted in each case in typical ringspot (figure 3C). One of the three plants was from seed of the Turkish plant (No. 5925) affected with ring-

Fig. 4. A lower leaf from Turkish tobacco plant 7823-16-1 affected with green ringspot thru the seed. The type of chlorosis shown seems to be a symptom of ringspot.

spot thru the seed the previous year. Thus one inoculation had served to infect three generations of plants with ringspot and the virus was present in two successive generations without the ring symptoms. Inoculations were made from the three plants when they were a few inches high, from about the eighteenth leaf of each, and from leaves at the top of the plants shortly

before blooming. Typical ringspot developt in each plant inoculated, in the course of about six days.

Fig. 5. Leaves of dark tobacco from plants affected with yellow ringspot thru seed, showing the leaf-edge chlorosis and necrosis following a period of four days of dull weather when the temperature of the house ranged from 49° to 76° F. Photographed April 27, 1931.

Throuout the growth of the three seed-infected plants there was nothing to indicate that they were affected with ringspot (figure 3B) altho their appearance was not normal. The older leaves gradually became chlorotic in small areas between the tertiary veins and, following blooming, this type of chlorosis spread well up the plants (figure 4). While it is not certain that this is a symptom of ringspot, it probably is, as it has not been noticed on plants not affected with ringspot, but has been observed on two other plants which had been inoculated with green ringspot. It appears to be a milder form of the bleach-

ing which develops on yellow ringspot plants. This symptom is not typical of any of the deficiency troubles as we recognize them. On one plant two leaves developt small, faintly chlorotic, areas in the growing point during the fall of 1930 but necrotic ring patterns were not observed on any of the three plants until September, 1931, when necrotic patterns were found in a tertiary sucker near the top of a sucker which had replaced the original stalk, cut back several months earlier.

While the number of known cases of seed transmission of green ringspot is small the fact that rings usually were not produced on the plants, as would probably have occurred if infection had been accidental, and the conclusive demonstration of seed transmission of yellow ringspot in the present studies, leave little doubt that seed transmission of green ringspot has been demonstrated. It is worthy of record that in the past five years the only ringspot which has developt in over 10,000 plants on which accurate records have been kept, on numerous other tobacco seedlings, and on many hundreds of tobacco plants used by others for physiological studies in the same house, has been on plants inoculated with the ringspot virus, or on plants, raised from seed of ringspot plants, which are recorded in this paper.

THE LEAF EDGE SYMPTOM OF RINGSPOT

Henderson (8) has demonstrated seed transmission of ringspot in Petunia but states that his studies with seed transmission in tobacco are not in agreement with those of the present writer (22). Henderson and Wingard (9) report that no ringspot plants were found in a total of 64,500 tobacco seedlings raised from seed from ringspot plants. These results are not surprising in view of the small number of plants in which the writer has demonstrated seed infection, as the typical ringspot symptoms usually do not develop in affected seedlings. If the leaf-edge symptom had not developt, these few affected plants with two exceptions might not have been detected. The leaf-edge symptoms result from injury to the tissues along the margins of the distal half of the leaf. It seems to occur if the young, rapidly expanding leaves are subjected to low temperature. The tissues

near the margins of these leaves become chlorotic and sometimes necrotic. As the leaf expands, it becomes somewhat distorted and has a pinched appearance (figures 5, 6). It seems

Fig. 6. A ringspot-affected Turkish tobacco plant which has been subjected to abnormally low temperature in the greenhouse. The leaf to the left shows the leaf-edge chlorosis and necrosis which develops in ringspot-affected plants under these conditions. Photographed May 1, 1931.

quite certain that this symptom develops only at relatively low temperatures (50° to 60° F.). It frequently occurs on tender leaves of suckers, during a cool period in the fall. It was at first taken to be a type of cold injury to supposedly healthy plants. Late in August, 1927, it was found on suckers of uncut tobacco on the Experiment Station farm. On August 26 the temperature was 52° F., the minimum for the month, and the average for the month was 5.1° F., below normal. This was one of the coolest Augusts on record. The symptom has not been observed in the field on suckers developt while the temperature is still relatively high. The few seedling plants in the greenhouse, with two exceptions, found to be affected with green

ringspot were discovered in the late fall before heat was supplied and while the temperature was relatively low. In the spring of 1931, while a considerable number of ringspot plants were present in the greenhouse, the glass of the house was whitewashed following a warm, bright period, when the day temperature ranged up to 105° F. This period of excessive heat was followed by a period of cool, rainy weather when the greenhouse temperature ranged between 49° and 76° F. for about a week. During this period many of the plants affected with both yellow and green ringspot developt prominently the chlorotic to necrotic leaf-edge symptom (figures 5, 6), but it did not occur on healthy plants or plants affected by other viruses. These instances suggest that studies on seed transmission of green ringspot should be conducted only under conditions where the temperature ranges from 50° to 65° F. for some time, before final observations are made.

COLD INJURY TO HEALTHY SEEDLINGS

The portions of the leaves of ringspot plants affected by cold, are the same as those of healthy tobacco seedlings which are sensitive to chilling. In 1929 and 1931 after the occurrence of prolonged cold winds, the small leaves of most of the plants in some beds were white and the larger leaves white and constricted along the margins, as illustrated in figure 7. This condition appears to be the result of the chilling of the young chloroplasts. While cold injury and the ringspot leaf-edge symptom are similar, ringspot plants are affected at a temperature not low enough to affect the leaves of healthy plants, or plants affected with any one of several other viruses.

SEED TRANSMISSION OF YELLOW RINGSPOT

Following the tests on seed transmission of green ringspot, two flats were sown, with the object of determining more accurately the extent of seed transmission of ringspot. One flat was sown with dark tobacco seed collected the previous fall from a green ringspot plant and the other from the plant of dark tobacco with ringspot and the bleached leaves. Soon after

germination, several chlorotic seedlings were noticed in the latter flat. These were allowed to remain until it was obvious that they would be overgrown by the green plants. They were

Figure 7. Cold injury to a healthy young white Burley plant in the plant bed, following cold, windy weather. Transferred to the tumbler for photographing.

then transplanted to a two-gallon jar (figure 8). Thirteen plants of a total of 878 (1.5 percent) were chlorotic. As the seed had come from a ringspot plant with chlorotic leaves it appeared probable that seed transmission of the virus had occurred. Subsequent inoculations from these yellow seedlings to Turkish tobacco plants resulted in typical ringspot of the yellow type as illustrated in figure 9. This demonstrated for the first time that a second strain of the ringspot virus existed.

At this time there were two Turkish plants in the greenhouse affected with ringspot and veinbanding. The inoculum had been collected from a Burley plant the previous summer by Dr. E. M. Johnson because of the peculiar appearance of the

plant in the field. Transfers to Turkish tobacco, then to beans and again to tobacco, dropped out the veinbanding virus, leaving only ringspot. Dr. Johnson had frequently stated during the

Figure 8. Photograph of 13 chlorotic seedlings raised from seed of a dark tobacco plant (7823 R 45) growing in the field. This plant had necrotic rings typical of ringspot and one leaf bleached nearly white. The younger leaves of the seedlings are green or green with a yellow tip. The older leaves are yellow or nearly white. These plants failed to develop any patterns typical of ringspot until more than 8 months old.

winter that this appeared different from other ringspots because of the greater amount of chlorosis. The plants (7795, 1 and 2) had been on the sill of the greenhouse, during the winter, with the tops against the glass. Seed had formed, but the seed pods were only about ¼ their usual size and contained few if any seeds. A flat was sown to these seed January 27, 1931, and a few days after germination chlorotic seedlings were numerous. In a total of 1,041 seedlings 156 (15 percent) were chlorotic (figure 10). A second flat was sown with the seed from 10 pods from the same source, February 14, 1931. The scarcity of seed

Figure 9. Yellow ringspot of Turkish tobacco produced by inoculation from chlorotic seedlings shown in figure 11, but at a later stage. A.—The typical necrotic rings common to both green and yellow ringspot, and a portion of a leaf, bordered by a necrotic pattern, showing yellowing typical of yellow ringspot. B.—A plant growing out of the necrotic stage. The upper leaves have yellow patterns and there is a mild general chlorosis of the tip leaves as contrasted with the dark green of the lower leaves.

is evidenced by the fact that from 10 pods only 1,380 seedlings developt, of which 236 (17 percent) were chlorotic (figure 11).

When the cotyledons of affected seedlings are first exposed to light they are green, but in the course of a few days they

Figure 10. Chlorotic seedlings and one healthy seedling raised from seed of plants 7795, 1 and 2, affected with yellow ringspot and veinbanding. The youngest leaves of the chlorotic seedlings are green or green with a yellow tip. The older leaves are yellow or white. Seed sowed January 27, transplanted February 13 and photographed March 7, 1931. When the healthy plant was 24 inches tall the tallest of the affected plants was 9 inches.

begin to bleach, finally turning nearly white. As each new leaf develops it is at first green but soon turns a golden yellow, beginning at the tip. The smallest leaves finally become nearly white (figure 10). As a consequence of the lack of chlorophyll, the affected plants grow slowly as compared with normal plants (figures 10, 11).

Transfers from small yellow seedlings with two or three true leaves, to Turkish tobacco, using 5 to 10 plants as inoculum, gave positive results in some instances, but symptoms were slow

in developing or were so mild as to be hardly detected. While occasionally necrotic ring patterns develop on rubbed leaves, the patterns were small and indistinct as contrasted with the

Figure 11. Photograph of a flat of seedlings raised from all the seed from 10 pods of Turkish tobacco plants 7795, 1 and 2 affected with yellow ringspot and veinbanding. All healthy seedlings were removed from the right half of the flat before photographing. The more rapid growth of healthy seedlings is evident. Seed sowed February 14 and photographed March 7, 1931.

usual necrotic spots and rings of green ringspot. Usually the first symptoms which appeared on rubbed leaves were faint chlorotic spots, followed in a few days by yellow patterns in the new leaves some of which became necrotic (figure 9). The virulence or concentration of the virus in these plants usually appeared to be increased as transfers from them to healthy plants nearly always develop severe necrotic symptoms on the rubbed leaves (figure 12). In these plants the disease was typical ringspot except that frequently the patterns were yellow.

Further tests of seed transmission of yellow ringspot were

conducted with the results given in table 1. Seed from 7 yellow-ringspot plants each produced chlorotic seedlings, totaling 1,175,

Figure 12. Differences in virulence of ringspot viruses. A.—Plant 8758-12 inoculated from plant 7823-16-1 infected thru seed with green ringspot. B.—Plant 8758-14 inoculated from plant 7823-15-3 infected thru seed with another strain of green ringspot. This difference in virulence seemed to be fairly well fixed. C.—Plant 8758-7 inoculated from a chlorotic seedling of plant 7795 (figure 8). D.—Plant 8758-2 inoculated from plant 7795-G which in turn was inoculated from a chlorotic seedling of 7795. Increase in virulence, or concentration of the virus, has resulted from passage thru one plant.

in percentages ranging from 1.5 to 17, with an average of 4.9 percent affected in a total of 24,060 grown.

The factor which determines the extent to which seedlings will be affected with ringspot has not been determined. From the evidence at hand it seems that the fewer the seed produced the higher will be the percent of infection. The pods of plant 7795 contained but few seeds while transmission was high, as compared with the other plants tested in which seed production was more abundant. In cross pollination studies more seed

resulted when pollen from non-ringspot plants was used than from self-pollinated plants. But the percent of seed infection was lower with normal pollen than with pollen from ringspot plants (table 1). This feature of the problem needs further study.

Table 1. Seed transmission of the yellow-ringspot virus of tobacco.

Origin of Seed	Source of Pollen	Total Seedlings	Seedlings With Yellow Ringspot	Percent Yellow Ringspot
Dark variety R 45	Self	878	13	1.5
Turkish 7795	Self	1041	156	15.0
Turkish 7795	Self	1380	236	17.0
Turkish 8278-B	Self	308	13	4.2
Turkish 8278-B	Non-ringspot plant	812	20	2.5
Turkish 7795	Self	1255	75	6.0
Turkish 7795	Non-ringspot plant	3000	89	3.0
Turkish 8278-B	Self	949	69	7.3
Turkish 8278-B	Non-ringspot plant	2400	11	.46*
Turkish 7795-L	Self	1027	16	1.5
Turkish 7795-M	Self	1747	47	2.7
Turkish 7795-N	Self	2275	113	5.0
Turkish 7795-W	Self	3322	157	4.7
Turkish 7795-V	Self	3666	160	4.4
		24060	1175	Av. 1.9

* Incomplete data.

YELLOW RINGSPOT IN THE PLANT BED

Only one test of seed from a yellow ringspot Turkish tobacco plant has been made in a plant bed out of doors. A few of the chlorotic ringspot plants were found scattered among the more vigorous healthy plants. At setting time, when the healthy plants were about 8 inches high, the ringspot plants were small and yellow and were hidden so that insects would hardly be likely to feed upon them and then spread the disease to other plants. It is doubtful, therefore, if tobacco seed plays any significant part in the distribution of yellow ringspot.

YELLOW RINGSPOT PLANTS SET IN THE FIELD

Yellow, ringspot plants of both Turkish and dark tobacco, from seed sown in January, were large enough by June to be set in the field. Growth during the summer was comparatively slow

and thruout the period the plants had an abnormal chlorotic appearance, altho the leaves contained considerable chlorophyll. The new leaves were usually a normal green color which bleached gradually to a yellowish white. The more exposed portions of the leaf and the thinner portions bleached most. The larger veins and the tissues immediately adjacent remained green. Suckers which developt in the fall and were not exposed to brilliant light remained nearly normal in color. No ringspot patterns were observed. The plants of both varieties produced normal-appearing flowers and the pods which developt appeared, on superficial examination, to be normal, but did not contain their full quota of seed, some having very few.

DISTRIBUTION OF YELLOW AND GREEN RINGSPOT

Green ringspot appears to be widely distributed in Kentucky in all tobacco-growing sections. During the summer of 1931, Mr. Russell Hunt, tobacco extension specialist, made observations in tobacco fields in 37 well distributed counties. Green ringspot was found in 96 of 98 separate crops. This seems evidence enough that green ringspot is well distributed over the tobacco growing areas of the State, that it undoubtedly has existed in Kentucky a very long time, and that the virus is now about as prevalent as it will ever be. Also this virus has been observed as one cause of cucumber mosaic wherever cucumbers are grown commercially in the State. As it does not cause "white pickle" of cucumbers it could well be the same as the disease discovered by Jagger (10) on cucumbers in New York.

Specimens of horsenettle (*Solanum carolinense*), with the same type of bleaching as occurs in tobacco plants affected with yellow ringspot and in potatoes affected with either ringspot, were collected in the experimental watermelon plots at Carmi, Illinois, during the 1931 tour of the Phytopathological Society. From one of these specimens the ringspot virus was transferred to Turkish tobacco. Its presence in that locality indicates that this virus may be looked for away from tobacco growing areas. Evidently the ringspot virus was present in Ohio 27 years ago,

for at that time Selby (16) published a photograph of what appears to be typical ringspot, which he called mosaic.

Ringspot of tobacco has been reported from New York, Maryland, West Virginia, Virginia, North Carolina, and Georgia (14), and the same virus in cantaloupe and pumpkin from West Virginia (14). Ringspot also appears to be present in tobacco in nearly all parts of the world where tobacco is grown (9).

Yellow ringspot has a wide distribution in the State, having been found many times in tobacco in Fayette County and once each in Todd, Daviess, Fleming and Nicholas Counties. The case in Nicholas County evidently had its source in the native vegetation, but that in Fleming County may have come from potatoes, as the veinbanding virus was present also in the affected tobacco plant. In 1930, altho it was not then recognized as such, yellow ringspot was abundant in suckers of Burley tobacco, on the Experiment Station farm, following harvesting, but in 1931 it was comparatively rare.

Table 2. The distribution of ringspot in Burley and dark tobacco in Kentucky in 1931.*

	No. of Plants Inspected	Ringspot	Percent	No. of Plantings
No. 5 Burley	272,665	233	.085	32
Burley	83,505	116	.14	50
Dark	20,190	246	1.22	42
Total in 96 fields	376,360	595	.16	

* The counts were made by Mr. Russell Hunt, Field Agent in Tobacco.

DISTRIBUTION OF GREEN RINGSPOT THRU TOBACCO SEED

Both ringspot diseases were exceptionally prevalent on the Experiment Station farm in 1930. No attempt was made to eradicate affected plants in a small seed plot of No. 5 Burley, a new root-rot resistant variety which was to be tested by farmers in 1931. Small lots of this seed were distributed to several hundred farmers with the understanding that a few rows of it were to be grown thru the middle of a field of their own tobacco. At the time this seed was distributed no thought was given to

the possible distribution of the ringspot virus. Early inspections showed that it was likely to be found in all plantings of No. 5 and it was found later in the 33 fields examined. Counts in other fields, however, raised the question as to whether seed was the primary source of the virus (table 2). While .085 percent of the plants in No. 5 plantings were found to be affected, the average infection in more than 1/3 of a million plants of all varieties was .16 percent. Burley varieties other than No. 5 contained .14 percent. Of the dark tobacco plants examined 1.22 percent were affected with ringspot. Seventeen direct comparisons of No. 5 and another Burley variety were inspected by Mr. Hunt. In a total of 213,500 plants of No. 5 examined, 182, or .085 percent, had ringspot while in 36,975 plants of other varieties growing in adjacent rows, 29, or .078 percent, were infected. In only one field did the counts suggest that the seed of No. 5 might have been responsible for the higher percentage of infection. In this field 11.2 percent of the 400 plants in the planting had ringspot while 2 percent of 100 plants of the local variety were infected. As all other plantings of No. 5 inspected had .6 percent or less (except one with 2 percent) this one case could hardly be attributed to seed. Most of the Burley seed planted in Kentucky is purchased from seedsmen, while most of that planted in the dark tobacco section is raised at home from a few selected plants. It seems that seed raised in a field known to contain many ringspot plants will cause no more ringspot than is found in the average field.

On the Experiment Station farm seed is not the primary source of infection. For three years seed raised in the greenhouse, for general plantings, did not diminish appreciably the amount of ringspot developing soon after setting. The distribution of ringspot, in years when it is abundant, is not of the random character to be expected from seed transmission. It suggests rather insect transfer from perennial plants. Furthermore, the presence of yellow ringspot in these plantings cannot well be attributed to seed transmission.

THE ECONOMIC IMPORTANCE OF TOBACCO RINGSPOT

If the virus of ringspot is spreading gradually thru tobacco areas and the number of infected plants is increasing each year, means should be taken to stop its progress as in some instances it is destructive to individual plants. If it is already widespread, as appears to be the case, and has been present in tobacco-growing areas for many years, perhaps it is already as injurious as it is likely to become. If so, extreme measures for eradication are not warranted.

Figures have already been presented which show the wide distribution of the virus in the State. Infection in 376,360 plants was only .16 percent with inappreciable injury to most of the affected plants. The highest infection was 11.2 percent in one field. In table 3 is shown the range of infection found in the planting examined by Mr. Hunt.

Table 3. Green ringspot in 127 plantings* of tobacco examined in 1931.

Percent of plants infected	0	Less than 0.2	.2—1.0	1.1—2	2.1—3	3.1—4	11.2
No. of plantings	2	36	60	21	4	3	1

* If two or more varieties were included in a field each was considered a separate planting.

Henderson and Wingard (9) report a decrease in yield of about 1 percent in the tobacco crop of Washington County, Virginia, in 1929, when ringspot was particularly severe. Fenne (5) reports a loss in the same county of 3.26 percent of the Burley crop, calculating that each affected plant is reduced in value 43 percent. In our observations in Kentucky this is far more injury than actually occurs except in rare plants. Burley tobacco is set close together and anything which tends to stunt one plant gives the next one an advantage and so it is not accurate to calculate loss from reduction in leaf area and plant size of affected plants alone.

PREVENTION OF RINGSPOT

Considering the limited infection and the small amount of

damage usually resulting to an infected plant, it is questionable whether control measures which involve any appreciable expense are warranted. The only measure of control which we are considering at present is the elimination of ringspot plants from crops which are being raised primarily for seed. The weed hosts are not well enough known to make recommendations for their eradication, even if it were desirable.

OVULE STERILITY CAUSED BY RINGSPOT VIRUS

Henderson (8) noted and illustrated partial sterility of petunias affected with ringspot. The same type of sterility occurs in tobacco plants altho it is not always evident, especially in plants fruiting in the field. Turkish tobacco plants grown in the greenhouse, affected by either yellow or green ringspot, produce fewer seed than normal. They bear an abnormally large number of completely sterile flowers, which usually dry up after the normal blooming period but do not fall off, in contrast with blossom drop on undernourished plants where abscission may occur at any stage from early bud to late bloom.

Ovule sterility appears to result from lack of fertilization. In some pods only the shrunken remains of the ovules are to be found. In others a few to many seeds are present, with many shrunken ovules. In occasional pods there may be a predominence of hollow seeds, the seed coats appearing to have developt normally but the embryo having failed to develop. At ripening time these seed coats wrinkle somewhat as they dry and thus can be distinguished readily from the normal seeds as well as from the shrunken embryos.

Great variation occurs in the degree of sterility between pods on a plant and between different plants. In table 4 is given a summary of counts made of the seeds contained in 258 pods from yellow and green ringspot plants. Most of the pods ripened in the greenhouse in May and June. Pods of the general size dealt with should contain from 1000 to 2000 seeds. A majority of pods contained less than 200 seeds.

Low temperature has at times appeared to be a factor in increasing sterility, but it is probably not the only factor. Plants

Table 4. The number of seeds per pod in 258 pods from green- and yellow-ringspot Turkish and dark tobacco plants.

Seeds per pod	0	1-100	101-200	201-300	301-400	401-600	601-1000	1001-1800
Number of pods counted	10	135	36	17	17	14	16	13

have produced small pods with few seeds under both low and high temperatures. It appears that pods which were pollinated following particularly cool periods in the greenhouse in the spring of 1931 were decidedly more sterile than those pollinated following more favorable conditions during this period and the remainder of the summer. During the cool period three Turkish tobacco plants which were under observation failed to set seed in 72 percent of 454 blossoms, while 96 blossoms which set seed contained an average of 91 seeds per pod. Normal pods on plants of equal vigor should contain at least 1000 seeds. Later in the spring, when temperatures were higher, a majority of the flowers on these plants set seed.

The number of seeds per pod is in inverse proportion to the number of aborted ovules which may be found clinging to the placentas of ripe pods (table 5). On a given plant there appears

Table 5. The number of seeds and aborted ovules per locule in pods collected September 14, 1931, from yellow-ringspot plants grown in a bench of soil in the greenhouse.

	No. Seeds	No. Aborted Ovules	Total
Locule 1.	610	682	1292
2.	683	296	979
3.	760	333	1093
4.	151	903	1054
5.	61	756	817
6.	647	514	1161
7.	64	982	1046
8.	397	634	1031
			Av. 1059

to be a direct correlation between pod size and the number of seeds which develop. An examination of the placentas of pods from healthy plants as compared with those from ringspot plants indicated that in the former shrunken ovules are usually

few while in the latter they are ordinarily numerous, even in well-filled pods. In some pods shrunken ovules are no more abundant than in pods from healthy plants. In poorly filled pods the shrunken ovules are closely packed, especially on the lower portion of the placentas.

POLLEN STERILITY

In seeking a possible explanation of ovule sterility, examinations were made of pollen grains of ringspot and healthy plants. On casual examination, pollen grains from ringspot plants appeared to be well filled with cytoplasm and to be about normal size. On more critical examination, the grains were seen to be of an uneven size and frequently more irregular in outline than normal grains.

Measurements of pollen grains from ringspot plants and from healthy plants or plants affected with other viruses or virus mixtures are given in table 6 and are shown graphically in Figure 13. The measurements of pollen from non-ringspot and from ringspot plants were always made under as nearly identical conditions as possible. A drop of 25 percent lactic acid was placed on a slide and mixed with a drop of a weak eosin solution in water. A drop of this liquid was placed at each end of a slide and ringspot pollen was mounted in one and non-ringspot pollen in the other. Measurements were made with a Spencer screw micrometer and a 4 mm Spencer objective. With this combination, one of the large units (4 turns of the drum) was nearly exactly the diameter of the majority of the normal grains. By placing a line of the large unit on one side of a grain its diameter in terms of the smaller units could readily be found with no further manipulation. Most of the counts were made of pollen from plants in a fair state of vegetative vigor, growing in ½-gallon jars. The temperature was considerably below optimum for tobacco during the period when the counts were made, except that from 7795 K. This was a vigorous, large-leafed plant growing in a bench of soil. Nearly all the flowers on this plant set large seed pods which appeared normal but contained an abnormally small number of seeds, and

averaged, in 17 pods which were ripe June 24, 1931, 480 seeds, but capable of containing 2,000. Ten representative pods harvested September 14 ranged from 400 to 1,200 per pod, with an average of 860 per pod.

The ringspot virus, whether of the yellow or green type, has a marked effect in retarding the development of pollen grains. Pollen from healthy plants and from plants affected with the

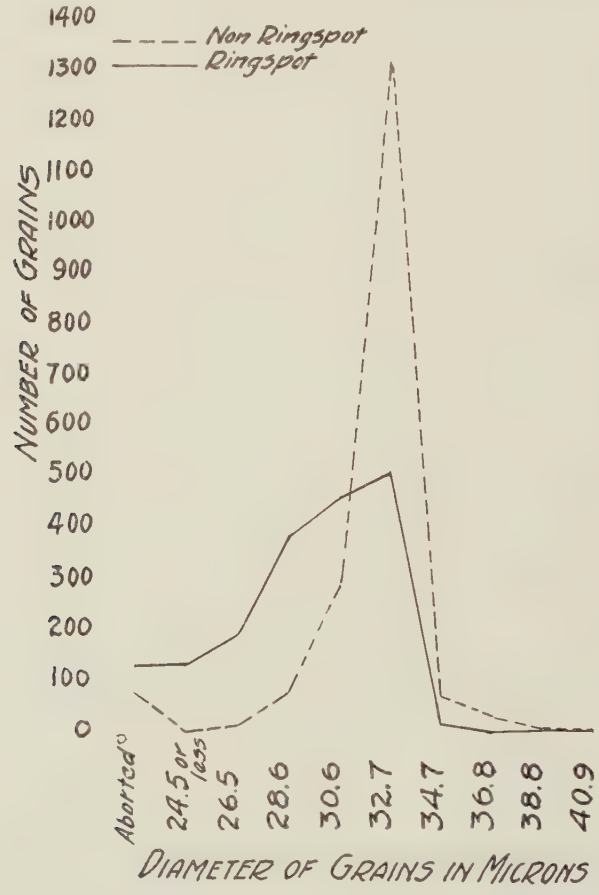

Figure 13. A graphic representation of diameters of 1800 pollen grains of Turkish tobacco from healthy plants or plants affected by viruses other than ringspot, and from ringspot plants.

Table 6. The range in size of 200 pollen grains from plants affected with green and yellow ringspot, and from healthy plants, and plants affected with other viruses.

Plant	Virus†	Collapsed	POLLEN SIZE MICRONE.*							
			21.5 or less	26.5	28.6	30.6	32.7	34.7	36.8	38.8	40.9
7823-15-2	Gr. R. S.	35	14	34	64	34	17			1	
Same		33	20	27	38	17	35				
7823-15-3	Gr. R. S.	25	16	18	15	22	12	2			
Same‡		26	2	50	10	31	22				
7823 16-1		17	12	15	59	65	2				
Same‡		32	4	16	37	55	56				
6907‡ (300 grs.)		21	4	20	73	56	105	1			
7795-K	Yel. R. S.	7	1	5	50	95	41			1	
7795	Yel. R. S. + V. B.	15	3	9	22	49	100	1			1
Same			27	27	78	51	47				
8278-B	Yel. R. S.		12	5	21	47	104	11			
Same		13	17	23	17	50	50				
	Percent	8.3	6.6	11.0	21.2	24.8	27.1	.7	.6	.04	.04
Healthy		23		1	3	10	110	18	2		
Same		28			2	19	144				
Same		3		1	3	23	170				
8376H.B.	H. P.	12		1	8	15	152	11	1		
8428 D	V. B. + H. P.	8			2	8	132	36	14		
8415-5-C	V. P. + H. P.	2		1	3	7	93				
Same	V. P. + H. P.	8		2	17	40	129				
	H. P. + ?	4			2	6	186	2			
8445	Tob. mos. and cuc. mos.	11			1	6	25	153	3	1	1
	Percent	4		.5	4.2	13.5	72.7	3.9	.9	.1	.1

* The figure represents the middle of the class.

† Gr. R. S. = green ringspot; Yel. R. S. = yellow ringspot; V. B. = vein banding; H. P. = healthy-potato; Tob. mos. = tobacco mosaic (severe); cuc. mos. = cucumber mosaic type 3.

‡ Not used in the graph.

delphinium virus,* vein-banding + healthy-potato, and severe tobacco mosaic + severe cucumber mosaic are uniform in shape and size as compared with those from ringspot plants measured at the same time. The majority of grains from these plants (72.7 percent) lie between 31.5 and 33.5 microns, whereas only 24.3 percent of grains from ringspot plants are of this size. The normal pollen grains (size class 32.7 microns, table 6) in the ringspot plants range from 9 to 53 percent, and are probably the ones which are functional.

In pollen development the stages up to the time of the liberation of the microspores from the tetrad appear to be normal.** Development is then normal for a time, as the pollen grain walls attain their normal markings and the cytoplasm greatly increases in volume. The uniformity with which the microspore nuclei divide has not been determined as the contents are not readily studied thru the pollen grain walls.

Often a factor that tends toward sterility in plants is more likely to affect pollen grains than egg sacs. This seems to be true of the ringspot virus, which suggests that pollen sterility is the principal cause of failure of seeds to develop. Tobacco flowers are normally self pollinated and small or large numbers of seeds develop, according to the viability of the pollen. Hand self-pollination does not appreciably increase the number of seeds. When pollen from healthy plants or from plants affected with other viruses is used the number of seeds is markedly increased. In table 7 are given the results of pollination experiments on five ringspot plants. In 63 self-pollinated pods there was an average of 114.2 seeds, whereas in 31 cross-pollinated pods there was an average of 1168.6 seeds. Further studies will be necessary in order to be certain that ovule sterility

* The measurements of pollen grains from the plant affected with delphinium virus and from plants affected with "ringspot", made at the same time, are not included in the table because another liquid was used, which swelled the grains somewhat more than the lactic acid-eosin solution. The "delphinium virus" grains were distinctly larger than the "ringspot" grains.

** But one instance was observed in which breaking down within the anthers of ringspot plants appears to have taken place before the late microspore stage. This occurred in the first flowers to bloom on 4 very vigorous plants of Turkish tobacco growing in a bench of soil (7795-K, etc.). The anthers contained few pollen grains of any kind and the flowers set from 4 to about 26 seeds each. While anther sterility sometimes occurs, in hermaphrodites, in the first flowers to bloom, as in the strawberry (18), it appears to be rare in tobacco and may, in this case, be due to the virus.

Table 7. Effect of self pollination, as compared with cross pollination from plants not affected with ringspot, on the number of seeds per pod.

Plant	Virus	No. Seeds in Self-Pollinated Pods	No. Seeds in Cross Pollinated Pods
7823-R15-2	Green ringspot	117*, 139, 149	1575*
		8	1908
		232, 366	1692
			1187
		249, 22	1783
7823-R15-3		17, 79	1077
		70, 184, 1, 146, 17, 200	1334
		94	1010, 1469, 448, 1008
		29, 46	1811
		45, 110, 14, 7	1200
8278-B	Yellow ringspot	215, 42, 164, 19	1260
		76, 155, 18	947
		343, 326, 83	1442
		24, 245	803
		30, 8	1085
		0, 12, 2, 0	1800**
		7, 21, 0	900
		0, 0, 0, 50	900, 1400
7795	Yellow ringspot + veinbanding		
		208, 58	343
		435, 5	960
		283, 480	615
		68, 64, 105, 138	869, 1202
7795-K	Yellow ringspot	150*	
		32	800
		350	1800
		331	1100
		307	500
	Average	114.2	1168.6

* Each group of numbers represents pods from the same branch, except in 7795-K.

** Counts expressed in even hundreds, were estimated by counting 100 seeds and then making piles, of approximately 100 seeds each, of the remainder.

does not result directly from the ringspot virus, but the present evidence suggests that pollen sterility alone is concerned.

DISCUSSION

The present studies show that two strains of the tobacco ringspot virus exist. The difference in the two strains seems to be in the effect which they have on chlorophyll, the one resulting in yellow patterns while the other results in green patterns in tobacco. Similar differences have been found in tobacco mosaics, in which there are green, yellow, and white strains, and in a group of viruses which affect cucumbers. These diseases are

characterized by ring-like chlorotic patterns which are green, yellow, and white in three strains, respectively. As "color" strains of the viruses are already well known, the discovery of the yellow ring-spot virus would have little significance were it not that it has given a rapid means of demonstrating virus transmission thru seeds, thus making further studies of this feature of the virus problem simpler.

While the writer recognized seed transmission of green ringspot before that of yellow ringspot, it is not so easily demonstrated. Plants affected with green ringspot may appear normal for long periods, and show none of the commonly recognized sysmptoms of the disease. Thus if these seedlings are grown under optimum temperature conditions, it may be difficult to know whether they are affected or not. Under lower temperatures, however, leaf abnormalities develop making recognition easy and certain. Other viruses fail to cause symptoms during certain periods of growth of the host plant. Tobacco plants affected with the typical cucumber mosaic virus may lose all symptoms for a time. Plants affected with the healthy-potato virus may lose all visible symptoms when the temperatures are high, but quickly develop severe necrotic symptoms when they are subjected to a temperature of about 50° F. Delphinium plants affected by a virus may show no signs of infection early in the season, but in the late blooming period may bleach out almost completely. Tobacco plants affected with the same virus may appear entirely healthy a short time after inoculation, especially if the initial symptoms following inoculation are mild. These facts should be kept in mind in any study of seed transmission of a vrius, as under optimum conditions for plant growth symptoms may fail to develop even tho the plant contains the virus.

As the green ringspot virus is transferred thru the seed, and as the affected plants grow normally enough to be set in the field, seed is an agent in the geographic as well as local distribution of this virus. It will be of interest to learn whether the geographic distribution of yellow ringspot is the same as that of the green strain. Whether seed infection is of any im-

portance in old tobacco-growing sections where the virus is already well established in weed hosts cannot be answered. Thus far the evidence suggests that seed transmission could be largely ignored as far as control measures are concerned on farms where the virus is already well established, as other sources of infection appear to be of greater importance.

While it is recognized that viruses frequently cause sterility or partial sterility in affected plants, sterility is often due to the abnormal development of flower parts, as in aster yellows, or to an upset in the normal metabolism of the plant, as in advanced peach yellows. In various legumes, affected with a virus, sterility more like that found in ringspot tobacco plants occurs, but in these plants growth usually is abnormal. Legumes affected by a virus and tobacco affected by ringsport, develop flower parts which appear normal, and in both partial sterility and seed transmission occur (4, 7, 12, 17). May it not be likely that seed transmission of a virus may be predicted from a knowledge of the effect of the virus on pollen development? This suggestion is based on the assumption that if a virus enters the egg sac it will also enter pollen mother cells and cause sufficient disturbance in some of the developing microspores to result in partial pollen sterility. That the presence of a virus in a pollen grain does not necessarily prevent its functioning is indicated by the work of Blakeslee on *Datura* (2). He demonstrated that a graft transmitted virus disease of Jimson weed was transmitted thru the seed of an affected plant and might also be present in as high as 100 percent of the seedlings of a normal plant pollinated with pollen from a plant which had recently contracted the disease. Plants which have had the disease a longer time, however, were usually completely pollen sterile, but otherwise fertile.

In tobacco, sterility does not appear to result from an effect of the virus on the sporophytic generation of the tobacco plant. Development of all flower parts, including the egg sacs, appears to be normal. Anther development is normal, and microspore development appears to be entirely normal until some time after the liberation of the spores from the tetrad. They are released

into a supposedly homogeneous anther sap where they may be considered to have begun a separate existence as the gametophytic generation. For a time after liberation, spore development continues to be normal. It consists in an enlargement of the spore, in an increase in cytoplasm and the laying down of the pollen grain wall. It is during the late stages of microspore development that irregularities take place resulting in pollen sterility. As all microspores in a locule appear to be subjected to identical environmental conditions it seems that difference in development must be due to conditions within the spore. Pollen sterility in this instance seems to be a result of the direct effect of a virus on the development of the gametophyte of a flowering plant.

When pollen from healthy plants is used on stigmas of ringspot plants seed infection occurs, but seemingly to a less degree than following self-pollination (table 1). Infected embryos develop normally, as they cannot be distinguished from healthy ones at the time of germination. Infection of the female gamete does not appear to cause extensive ovule sterility, as cross-pollination frequently resulted in nearly the maximum quota of seed per pod (table 7).

With the conclusive demonstration of seed transmission of ringspot thru both petunia (8) and tobacco seed the question as to the possibility of embryo infection by other viruses of the Solanaceae naturally arises. The possibility of transmission, without symptoms being evident in the seedlings, must be considered as there are several virus diseases of tobacco the symptoms of which are suppressed for varying periods of time. Bewley and Corbett (1) have presented evidence which they interpret as proving that mosaic disease of both the cucumber and the tomato are transmitted by seed. They state that seed transmission (presumably of typical tobacco mosaic) also occurs in tobacco. Because there are so many viruses capable of causing a mosaic disease in either cucumbers, or tomatoes and tobacco, one would not wish to question their interpretation of the results as reported. However, in the past 9 years the writer has pulled and examined many thousands of tobacco plants from

beds, many of which were sowed with seed from plants affected with typical tobacco mosaic. Thus far, when men who chew or smoke natural leaf tobacco have been kept away from the beds until pulling time, not a single plant affected with typical tobacco mosaic has been found. In more limited observations the same applies to tomatoes. If observations of this type are not considered conclusive, there is further evidence from the field counts of mosaic tobacco plants, taken usually about three weeks after setting time. With reasonable precautions to prevent infection from the hands of pullers (who chewed tobacco but were given sterilized tobacco while pulling) mosaic was found about 3 weeks after settings, in only .44, .05, .02, .01, .16, and .007 percent of from 13,000 to 66,000 tobacco plants set on the Station farm each year in the years 1926 to 1931, respectively. Less than 2 mosaic plants are present in an acre of Burley which has .02 percent infection. It would be supposed that if seed transmission of mosaic occurred, a few instances might be expected where a puller had pulled a diseased plant and then transmitted the virus to several plants pulled subsequently, but no such instance has been observed in the six years. The figures given have value in the problem of seed transmission of typical tobacco mosaic, as each year seed from at least 50 different plants, and often more than 100, were sowed, many of them having had mosaic resulting from handling incident to selection, bagging, hybridizing, etc. It seems safe to conclude, therefore, that seed transmission of the typical tobacco mosaics occurs so seldom, thru tobacco seed, as to be of no practical importance whatever.

As to seed transmission of other viruses of tobacco, a slight amount of evidence has been obtained that a virus found quite commonly in cultivated delphinium in this region may be transmitted thru both tobacco and delphinium seed, as affected plants have been found in plant beds of both. Aside from this no evidence whatever has been obtained of seed transmission of the other tobacco viruses.

SUMMARY

1. The ringspot disease of tobacco is as widely distributed in Kentucky as typical tobacco mosaic and much more widely than other virus diseases of tobacco. The same virus causes a disease of cucumbers and potatoes. *Solanum carolinense* may carry the virus from year to year.

2. Two strains of the ringspot virus have been distinguished; one causing patterns of various shades of green, and the other causing yellow patterns in tobacco. Both types occur in tobacco and have been transferred from naturally infected potatoes to tobacco.

3. Both ringspot viruses are transmitted thru seed from affected plants. Seedlings affected with the "green" strain may not show symptoms unless subjected to abnormally low temperature. Seedlings affected with the "yellow" strain bleach to a yellow color a few days after germination. Seed infection with yellow ringspot has ranged from about 1 to as high as 17 percent.

4. A leaf-edge symptom developing on ringspot plants subjected to low temperature is described and its similarity to cold injury of healthy plants pointed out.

5. Seedlings infected with the yellow ringspot virus grow slowly and could hardly be set in the field. Those affected with the green ringspot virus grow rapidly and could be set in the field where they might act as sources of infection for other tobacco plants and for native vegetation. It is probable that tobacco seed has been a factor in geographic distribution of the green ringspot virus.

6. The ringspot viruses at present cause but slight injury to tobacco and as they have undoubtedly been present in the State for many years are not likely to become increasingly injurious.

7. Seed production on ringspot-affected plants is abnormally low. This appears to be due to partial pollen sterility which seems to result from microspore infection with the virus. Seed production is markedly increased thru the use of pollen

from healthy plants or plants affected with viruses other than ringspot.

8. Microspore development, on ringspot plants, appears to be normal for some time after liberation from the tetrad. The walls develop normally and the cytoplasm increases in volume. A majority of grains are, however, below normal size when the anthers are mature.

9. Five other viruses, alone or sometimes in combination, appeared to have little or no effect on pollen development.

10. The suggestion is made that a study of pollen development on an affected plant may indicate whether or not embryo infection is likely to occur with a given virus.

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2. A Virus Disease of Delphinium and Tobacco.

A virus disease of the garden varieties of perennial delphinium is prevalent in the vicinity of Lexington, Kentucky. The symptoms are strikingly similar to those of tobacco ringspot, altho the disease is caused by a distinct virus. There are chlorotic ring patterns on individual lobes (figure 14, A) and sometimes patterns which extend into each of the lobes of a leaf, following more or less closely the primary veins (figure 14, B). The patterns may or may not be present as the plants start growth in the spring but are likely to become more prominent as the leaves become older. Following bloom a few or all of the leaves may bleach to a light greenish yellow similar to the bleaching of yellow ringspot of tobacco. Whether the disease in this region is in any way a cause of dying following bloom, has not been determined. From a limited number of observations it appears that the extensive chlorosis occurring at this time may be a factor in the failure to produce a healthy, normal growth after death of the flowering stalks.

The disease has been observed in several gardens in this vicinity. It has been present in most of the delphinium plants growing in a local nursery and was present in approximately 25 percent of the seedlings of Belladona and Bellamosum varieties in a seed bed when the plants were only a few inches high, but appeared in only 2 plants in a lot of English hybrid seedlings only a few feet away in the same bed. Dr. L. O. Kunkel informed the writer that the disease is quite common on delphinium in the vicinity of Yonkers, New York.

TRANSMISSION OF THE DELPHINIUM VIRUS TO TOBACCO

In June, 1930, while affected delphiniums were making rapid growth, inoculations were made from them to Turkish tobacco plants growing in the greenhouse. In the course of four or five days symptoms became evident in the form of necrotic ring and line patterns of various shapes (figure 15). These

Figure 14. Leaves of delphinium plants affected by a virus. The symptoms are similar to ringspot in tobacco but the disease is caused by a different virus. Natural infection.

symptoms appeared identical with the early symptoms of coarse etch of tobacco.*

Figure 15. Turkish tobacco leaves from a plant (6484 B) inoculated with crushed leaves from a virus-affected delphinium plant.

A doubt of the correctness of this diagnosis has arisen, as the host range of the two viruses has been found to differ. The delphinium virus transfers readily to cucumbers and may be transferred to tomatoes, but less frequently, whereas the coarse etch virus does not seem to go to these plants.

The symptoms of the delphinium virus in Turkish tobacco are variable. The earliest symptoms in the inoculated plants are usually fine ring and line necrosis on the rubbed leaves (figure 15). If necrosis is very slight, the subsequent leaves may appear to be entirely normal or may have only occasional faint chlorotic line patterns, only evident when the leaves are examined by transmitted light. If the necrotic patterns are more extensive on the rubbed leaves, the next leaves may be distorted as the result of extensive necrotic line patterns and chlorosis. Subsequent leaves may be normal, or small, light

* Kentucky Agr. Exp. Sta. Bul. 306. See pp. 300 to 302.

green, and slightly distorted, with here and there small, necrotic line patterns. The general appearance is more that of plants affected with one of the mild cucumber mosaics than with any of the typical tobacco mosaics. Later, on the older leaves of some of the inoculated plants, somewhat chlorotic areas

Figure 16. The delphinium virus in tobacco showing dot and line necrosis, and the "scarified" symptom on one leaf. The youngest leaves appear normal.

develop with a peculiar scarified appearance (figure 16). While not always present, this symptom seems to be typical of this virus, as it has not been observed on plants affected with any of the other viruses studied. The delphinium virus transfers from tobacco to delphinium by the rubbing method. Typical symptoms appear in two or three weeks on a leaf or two, but may not be present on subsequent leaves in plants growing in the greenhouse in winter. The virus may be transferred from apparently healthy leaves of affected delphinium to tobacco where it again produces the necrotic line disease.

TOBACCO, A NATURAL HOST OF THE DELPHINIUM VIRUS

The delphinium virus not only causes a disease of tobacco

when artificially inoculated but the disease occurs in tobacco growing in the field, both in Kentucky and in Minnesota. In 1930 collections of this virus were made in Burley and Turkish

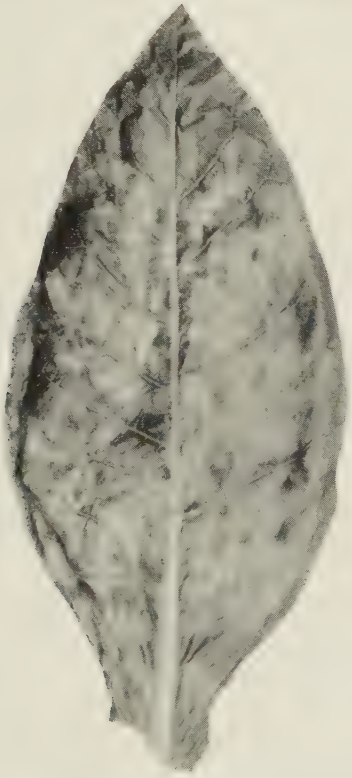

Figure 17. The delphinium virus in white Burley tobacco grown on University Farm, St. Paul, Minnesota.

tobacco plant beds on the Station farm at Lexington. A few collections were made also in the field, from tobacco plants affected with a virus disease differing from any recognized at that time. The virus from the beds and from the field, when transferred to tobacco plants in the greenhouse, produced a disease quite similar to the delphinium virus disease in tobacco.

Thru the cooperation of Dr. J. G. Leach and Mr. James Walter, of the Division of Plant Pathology and Botany, Univer-

sity of Minnesota, White Burley tobacco plants were grown in 1930 on the University Farm, St. Paul, in order to determine what viruses would be present in tobacco on this farm where potatoes rapidly degenerate. Specimens from 52 affected plants were sent to the writer for study and in many of these a virus was present, either alone or mixed with the veinbanding virus, which seemed to be identical with the delphinium virus collected from tobacco and delphinium in Kentucky. While the early symptoms on inoculated Turkish tobacco plants appeared to be identical with those of coarse etch, the symptoms on the leaves received from Minnesota were distinctly different from coarse etch as it appears on field-grown tobacco in Kentucky. The affected leaves of White Burley tobacco from Minnesota had irregular, ring-like, orange-yellow, chlorotic patterns scattered over the leaves. Sometimes these were bordered by a fine, necrotic line or by necrotic dots (figure 17). Altho the patterns were rather suggestive of tobacco ringspot, the two would not be confused by one familiar with tobacco ringspot. The Minnesota virus was transferred to delphinium where it produced results indistinguishable from those induced by the virus originally obtained from delphinium. It was transferred to tomato and cucumber, in each of which infection occurred and became systemic. The frequency with which the delphinium virus was associated with the vein-banding virus in the collections of tobacco from Minnesota suggested that one of its hosts might be the potato. Inoculations to potato seedlings have not yet confirmed this view.

CUCUMBERS, A HOST OF THE DEPHINIUM VIRUS

The viruses obtained from dephinium, tobacco plant beds, from a tobacco field in Kentucky, and from the Minnesota collections all transfer quite readily to cucumbers where a conspicuous mosaic disease results. Inoculation to cucumbers was used as a means of separating this virus from the veinbanding virus in the Minnesota collections. The disease differs in some ways from that caused by certain other virus diseases of the cucumber. In the virus collection at the Kentucky Experiment

Station there are at least 9 viruses which will cause mosaic in cucumbers, each of which produces a distinct disease in tobacco. But the symptoms in cucumbers are not readily distinguished. In a limited number of transfers from naturally infected mosaic cucurbits the delphinium virus has not been found.

SEED TRANSMISSION

The appearance of numerous affected plants of two varieties of delphinium (Belladona and Bellamosum) in a plant bed and the nearly complete absence of infection in another variety (English hybrids) a short distance away suggests seed transmission of this virus. As older affected plants were not far distant, insect transmission could have occurred. The presence of affected plants in plant beds of white Burley and Turkish tobacco likewise suggested seed transmission, as plants in the bed rarely are affected with anything but the typical tobacco mosaic and occasionally with ringspot.

Seed transmission studies of the delphinium virus thru delphinium seed and thru tobacco seed have so far failed to give positive results; but negative results do not necessarily mean that transmission does not occur, as this is one of the viruses which, at times, produces no visible symptoms either in tobacco or in delphinium. Consequently, infected plants of either might not be recognized. Until a means is known of causing the development of symptoms in symptomless carriers, as can now be done for ringspot of tobacco, seed transmission studies may be futile.

CLASSIFICATION OF THE DELPHINIUM VIRUS

No classification of plant viruses has yet been worked out which is satisfactory. Nevertheless the viruses which affect tobacco may be grouped on the basis of their ability or lack of ability to withstand drying, on similarity of host range, and on similarity of symptoms. The delphinium virus has little in common with tobacco mosaic, as it differs in symptoms and host range and has not the ability to withstand drying. It differs from the etch viruses and veinbanding in having a wide host

range, whereas these appear to be limited to the solanaceae. Apparently it differs from tobacco ringspot in not affecting pollen development or causing sterility, but is similar to tobacco ringspot in having a wide host range and in the similarity in patterns. The delphinium virus corresponds most closely to the typical cucumber mosaic viruses, and to a group of other viruses capable of causing mosaic in cucumbers, in their similarity of necrotic ring and line patterns on inoculated leaves, and in their wide host range including plants in distinct orders, and in the general symptoms on the plant as a whole.

SUMMARY

A virus disease of the garden varieties of delphinium is prevalent in the vicinity of Lexington. The virus transfers readily to tobacco and apparently is identical with a virus sometimes found in White Burley tobacco, both in Kentucky and at University Farm, St. Paul, Minnesota. The delphinium virus appears to affect plants in three families (Ranunculaceæ, Solanaceæ and Cucurbitaceæ) in two orders (Ranunculales and Campanulales) of the flowering plants. It is possible that this virus may be seed-borne, but as yet this is undetermined.

3. A Virus Disease of Plum and Peach.

During the past six or eight years plum trees (*Prunus* sp.) in the Experiment Station orchard have been dying from an unknown cause. The trees gradually lose vigor. The young leaves in the spring are light green, narrow, and curled under (figure 18). They gradually take on a more normal shape but are distinctly chlorotic thruout the summer. The larger branches gradually die, or become so weak as to make but little growth, but vigorous water sprouts may grow from near the lower crotches. Usually these appear entirely normal except for patterns on an occasional leaf (figure 19). The fruit varies in quality from year to year, sometimes being insipid and small while at other times the quality is fair. When the trees are in the condition of those shown in figure 18 they produce few fruits. Except for the general devitalized condition of the tree, which may result from various causes, the abnormal spring foliage, and the presence of occasional leaves with distinct patterns of the virus type, there are no symptoms by which the disease can definitely be diagnosed.

HISTORY OF THE DISEASE

The plum orchard in which this disease occurred is adjacent to a peach orchard. In 1925 June Elberta (tree 108) developt a few "bumpy" peaches, fruits with raised dark red spots which ripened earlier than the remainder of the fruit (figure 20). The tree is still living (1931) altho not vigorous. The fruit is insipid in some seasons, but in 1931 was of fair quality. Each crop year the tree has produced a few of the bumpy fruits.* While the foliage showed no very conspicuous symptoms on casual inspection, many leaves were present which had chlorotic

* The bumpy peaches were called to the attention of Professor C. W. Mathews, who sent specimens of them to W. F. Rofkar, Wooster, Ohio. In reply, Mr. Rofkar stated that the trouble was "frequently associated with trees of low vigor and not necessarily affected with yellows." He further states, "this characteristic spotting has been observed in peach orchards in northern Ohio for a number of years. However, we have never been able to definitely associate it with peach yellows."

and necrotic markings of no very definite pattern (figure 21). Bumpy fruits were found also on a Rivers tree (B 30, H.) and on an Illinois (D 20, S. B.). The Superintendent stated that

Figure 18. Twigs from Red June (429), Station orchard, showing severe and moderate symptoms. This tree was affected by a virus as indicated by transfers to peach. Photo April 19, 1930.

he had seen bumpy peaches on J. H. Hale the previous year. Since 1925 yellows has developt in a number of trees in this orchard and in 1928 in a small block of young Elberta trees about 200 yards away. In each case the first fruits to show symptoms of yellows also showed the bumpy condition. In both orchards, trees have produced an occasional bumpy fruit but have not since developt symptoms of yellows, this suggests that the two diseases may result from different causes and that both may be present in the same tree.

It was with the purpose of determining whether or not plum trees which were dying were affected with the peach yellows virus that inoculations were made from them to seedling

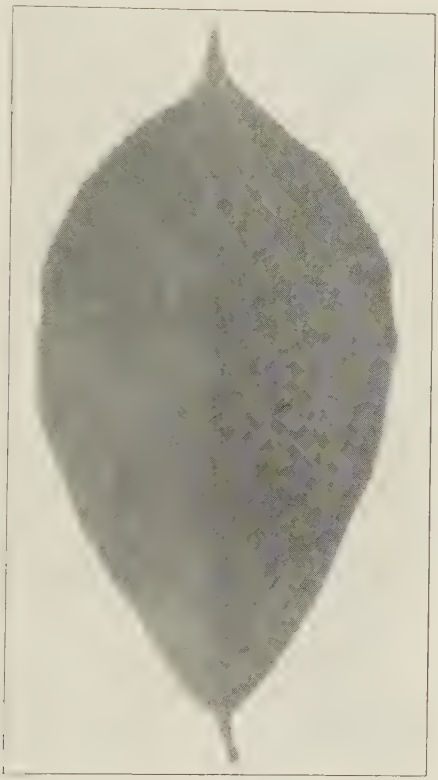

Figure 19. Leaf from a water sprout of Abundance 422, with chlorotic pattern. Frequently the portion of the leaf "within" the pattern is lighter colored than that without.

peach trees by the budding method. For comparison, seedling peach trees also were inoculated with typical peach yellows and with the June Elberta tree bearing occasional bumpy fruits. Discovery of the virus disease of plums herein described was a result of these inoculation studies.

1929 BUDDING EXPERIMENTS

In June, 1929, 120 peach seedlings from pits planted that spring, were budded with material from 11 plum trees, and from 6 peach trees, 3 of which were known to have yellows.

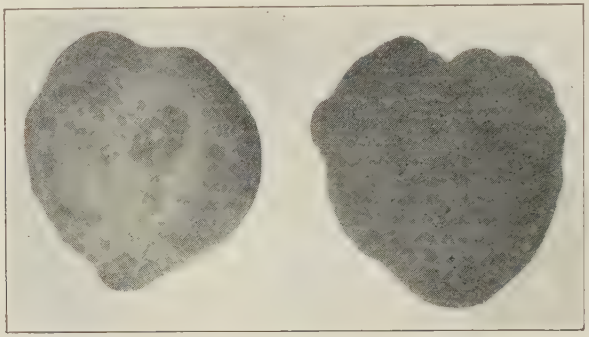

Figure 20. Left—a bumpy peach from June Elberta (108). Photographed July 3, 1931. Right—from Hiley (107). Photographed August 8, 1928.

From 5 to 9 trees were budded with each variety. Tho the bark of the "bud" united with the peach tree in most cases, usually the bud itself did not grow hence most of the records were of peach foliage.

The following spring, April 19, 1930, 10 months after budding, two lots of peaches budded with Abundance (422) and Red June (429), respectively, had chlorotic ring and line patterns, of the type shown in figure 22, on a few leaves. Three of 5 trees budded with Red June, and 8 of 9 trees budded with Abundance had these patterns. By July 21, 3 of 5 remaining trees budded with Bovay (409) showed distinct patterns, and 3 of 6 trees budded with Shiro (502), and 1 of 6 trees budded with Shiro (501) showed distinct patterns in a few leaves. The remaining trees budded with plum, while at times considered suspicious, never developt unmistakable symptoms. During 1930 and 1931 leaf symptoms were the only means by which affected trees could be detected. The virus appeared to have no effect on the vigor of the peach trees.

Of the peach trees budded with peach, none showed any symptoms except those from two trees known to have yellows.

Five of six trees budded with Edgemont (120) and the four remaining trees budded with R3T1, both of which had clear-cut cases of yellows in the orchard, developt willowy shoots from near the ground. These were typical cases of yellows and could

Figure 21. Leaves from June Elberta (108), a tree which has produced a few bumpy fruits each crop year since 1925. The same type of symptom sometimes is seen in unsprayed budded nursery trees suspected of harboring the causal agent of bumpiness. Photographed July 3, 1931.

not be confused with the disease from plums. No symptoms were noticed on the peach trees budded with peach wood from trees producing bumpy peaches. These experiments demonstrated that at least 4 plum trees in the orchard were affected with a virus which is transmissable to peach and which is distinct from the peach yellows virus.

1930 BUDDING EXPERIMENTS

On June 6, 1930, inoculations by budding were made from 8 varieties of plums, each to 3 two-year-old peach seedlings, and from June Elberta (108), Hale, and R3T1 peach trees, the two

latter having yellows. May 8, 1931, patterns were present on the peach foliage of the 3 trees budded with Red June (429) of 3 budded with October Purple (431) and on 2 of 3 budded with Abundance (422). On the trees budded with October Purple

Figure 22. Leaves of a peach seedling budded with Abundance (422) plum. Photographed May 9, 1931.

the cholortic patterns were frequently small and became necrotic, the tissue enclosed in the pattern being shed, making ragged leaves. The remaining trees budded with other varieties of plum appeared healthy. Some of the trees on which buds from yellows trees were placed had developt the typical willow growth of yellows by fall.

Both years buds from some plum varieties with the type of growth shown in figure 18 failed to cause patterns in peach foliage even when unions occurred. These results may be interpreted in two ways. The symptoms in the plum trees in

the orchard may not be caused by the virus which causes patterns in the peach foliage. Or, the virus may be the cause of the symptoms in the unhealty plum trees, but it may not be virulent enough to cause symptoms in the peach trees to which the virus

Figure 23. Abundance (422) leaf from a tree budded in 1929. Photographed May 9, 1931.

is transmitted. The latter hypothesis appears to be the more plausible for these reasons. In both the 1930 and the 1931 budding tests marked differences were evident in the prominence and persistence of the leaf symptoms in the inoculated peach trees. In the 1929 tests, patterns were numerous and prominent on trees budded with Red June, were less so on trees budded with Abundance, while they were scarce and often difficult to find on trees budded with Bovay and Shiro. In the 1930 tests the symptoms were most numerous on trees budded with October Purple (431) (not used in 1929), somewhat less prominent on those budded with Red June and still less so on those budded with Abundance. Considering that the tobacco ringspot virus and the delphinium virus often fail to produce recognizable symptoms in affected plants, it is not unreasonable to suppose that the plum virus, after long habitation in certain

varieties, might fail to produce recognizable symptoms in the leaves of a new host to which it has been transferred.

Figure 24. Abundance plum leaves, from a nursery, with prominent yellow patterns. Photographed May 14, 1931.

PATTERNS ON PLUM LEAVES IN THE ORCHARD AND NURSERY ROW

After virus transmission from plums to peaches had been demonstrated, patterns were looked for on plum leaves in the orchard. They were first found on a few leaves borne on vigorous water sprouts of Abundance (422) (figure 19). Later they were found on chlorotic leaves on October Purple (430), and Red June (429). Without careful inspection of many leaves, with transmitted light, the patterns on the leaves of these trees would not have been found. The trees had the same general appearance as other diseased trees on which patterns were not found and which failed to transmit patterns to peach seedlings to which buds were transferred. While a few patterns were found on peach trees budded with Shiro, no patterns were found on Shiro leaves in the orchard.

Of the plum buds which grew on peach trees a few produced good growth and on the leaves of some of these prominent patterns were found (figure 23). On Abundance the patterns on the lower leaves were lemon-yellow and could be clearly seen 20 feet away. A few patterns were also found on Red June leaves.

THE PLUM VIRUS IN A COMMERCIAL NURSERY

With the assistance of the owners, the budded plum and peach trees in a nursery were examined on October 2 and 3, 1930. A row of Abundance plums presented a peculiar appearance. Nearly all trees in the row had recently made from a few to eight inches of terminal growth, while nearly all trees of other varieties in adjacent rows, except Endicott, had formed a terminal bud for passing the winter. The new Abundance and Endicott leaves were light green, narrow, and rolled downward, presenting much the same appearance as diseased trees in the Station orchard. The plums were all budded on seedling peach stock. Peach sprouts were growing from the root stocks of some of the trees. Of 74 sprouts examined in the Abundance row, the leaves of 39 had prominent ring-and-line patterns of the type shown in figure 22. In an examination of peach sprouts in rows budded with Burbank, Endicott, Damson, *Prunus triloba,* flowering almond, and several varities of peach, no patterns were found.

On May 14, 1931, another block of budded trees in the same nursery was examined. The buds had been taken from trees in the block just discussed. At this time the trees were about 12 to 18 inches high. Patterns were prominent on the leaves of some of the Abundance trees, in the form of concentric bands of yellow and green tissue or large areas of yellow tissue. Frequently the patterns were recognizable at a distance of 20 or 30 feet (figure 24). Two hundred and twenty-two of these trees were examined, of which 57 appeared to be without leaf patterns, 89 had faint patterns and 76 had prominent patterns similar to those in figure 24. Patterns were not found in other varieties of plums or peaches.

In 1930 an extensive examination was made of the peach sprouts coming from the root-stocks of trees, budded with peach, in the nursery. No patterns were found. In an examination of the trees, however, an occasional small leaf was found with distinct chlorotic ring-and-line patterns. In a few the tissue surrounded by chlorotic patterns was excised giving a shot-hole effect. Leaves were saved and a year later the patterns could still be easily seen. Groups of trees, evidently propagated from a single bud stick, had started into growth late in the fall in the same way as the Abundance trees, while on the adjacent trees the terminal bud remained dormant. While the evidence was not at all conclusive the plum virus appeared to be present in at least some of the Elberta trees.

The system of budding, whereby bud sticks were taken from trees budded the previous year, had been practiced for about 3 years preceding 1929. Consequently the diseased Abundance trees had been adjacent to a row of Burbank trees continuously during this time. There was no positive evidence that transmission had occurred. It is possible, however, that the virus when present in certain plum varieties is affected in such a way that it does not produce patterns in the peach to which it is transferred. In the Station orchard, Burbank trees which appeared to be affected by the virus failed to produce symptoms in budded peach trees.

SPREAD OF THE VIRUS IN BUDDED TREES

Evidence on rapidity of spread of the virus in budded trees was obtained by budding two-year-old peach trees near the ground and at various heights from the ground and observing the distribution of leaves with patterns the following year. Trees budded near the ground line were found to have patterns in the leaves 3 feet or more above the point of insertion of the bud and trees budded 2½ feet above the ground were found to have patterns in the leaves on shoots originating below the ground line. The evidence indicates that in a period of 11 months the virus can spread either upward or downward for

at least three feet and it is probable that in that time it spreads thruout the tree.

Figure 25. A ringspot-like virus disease of *Malus* sp.

SIMILAR VIRUS DISEASES OF THE APPLE AND ROSE

A virus disease of apple (*Malus* sp.) similar in leaf symptoms to that of the plum has been found on the Experiment Station farm in a group of four apple trees planted as ornamentals north of the horticultural greenhouse. The writer has watched these trees for several years but it was not until the spring of 1931 that the virus symptoms were noticed. On three trees of one species the leaf symptoms were very prominent during the entire season (figure 25). The patterns consisted of chlorotic rings and lines of various shapes similar to those on the peach leaves and of the same character as the ringspot patterns on tobacco leaves except that no necrotic patterns were observed on the apple. This species of apple has leaves as shown in figure 25, and fruit which is green, very bitter in taste, about 2 inches in diameter across the core, less than 1 1-4 inches from

the pedicel to the base of the calyx lobes, and has a pedicel nearly 1 3-4 inches long. It is evidently an introduced species. The other tree was of another species of *Malus* with bright red blossoms and red flesh. The leaves of this tree appeared to be

Figure 26. A ringspot-like virus disease of climbing rose.

affected but did not have the prominent patterns of the other species. This tree died from no apparent cause in the summer of 1931. Patterns have not so far been found on the leaves of apple varieties in the orchard.

A virus disease of rose (*Rosa* sp.) was first observed by the writer in his garden in a hybrid tea rose, the variety of which was not recorded. It was later seen in a planting of hybrid perpetual roses on the Experiment Station grounds and was found in a climbing rose, behind the Experiment Station building, which was more than 12 years old. The patterns on the leaves of the bush roses were indistinct because of the thickness of the leaves but where found were typical of the ringspot type of disease. On the leaves of the climbing rose the patterns were distinct and readily seen only on the lower leaves partially

shaded by grass and weeds (figure 26). This disease seems to be different from the rose chlorosis described by White.* Specimens of the latter sent the writer by Dr. Freeman Weiss do not show the prominent ringspot-like patterns, and the ringspot-like rose virus does not appear to cause leaf distortion. A single plant affected by rose chlorosis (of White) was observed by the writer in a garden in Lexington and it differed greatly from ringspot-like disease.**

Whether there is any relation between the diseases of the plum, rose, and apple cannot be stated. There is a marked similarity in patterns produced by the viruses on the three hosts and there is a possibility that they may be identical as the three hosts are members of the Rosaceae.

DISCUSSION

The evidence indicates clearly that there is a virus disease of plums in the Experiment Station orchard and in a nearby nursery. There is also a condition in certain peach trees both in the old Station orchard and in a younger block of Elberta peaches, planted with trees from the above-mentioned nursery, which results occasionally in what has here been called bumpy peach. Bumpiness occurs on an occasional fruit on otherwise healthy-appearing peach trees, more often being produced on short spurs on the larger branches or trunk. Trees which have produced bumpy fruit in previous years usually lack vigor and increase trunk diameter slowly. On trees which are in a vigorous condition and contract yellows the bumpiness may be very marked. This condition has not been described as a symptom of yellows as far as the writer is aware and, as it occurs on trees which do not have yellows, as indicated by observation over a period of years and by budding experiments, the suggestion is here made that bumpiness is caused by a distinct virus. There are no very typical leaf symptoms altho the symptoms shown in figure 21 may be the result of the virus. The same symptoms

* White, R. P. An infectious chlorosis of rose. Phytopathology 20, 130, 1930.

** Since this was written another paper by White (Phytopathology 22, 53-71) has been received in which patterns similar to those here pictured are illustrated.

have been found in the nursery on unsprayed peach trees. The effect of the virus on the tree, if any, has been to gradually reduce its vigor. The June Elberta tree under observation is becoming less vigorous each year altho well cared for. The young peach orchard altho planted in rich garden soil is not growing according to expectations. When first set it made vigorous growth.

The nearly complete absence of the ringspot-like patterns on the leaves of the peach trees raises a question as to relation between the plum virus and this condition in the peach. A few leaves on Elberta trees in the nursery were found with what the writer considers unmistakable patterns of a ringspot-like virus.* The tendency toward a disappearance of patterns during the summer and the variation in extent of pattern production on recently budded trees suggest that habitation in certain varieties may affect the virus to the extent that it does not cause patterns. The whole question of the effect of the plum virus on peach varieties and its identity with the factor which causes bumpy peaches needs further study. A cumulative effect of a virus is probably to be expected and may explain the gradual failure of affected plum trees and lack of vigor in the suspected peach trees.

SUGGESTIONS FOR ERADICATION OF THE VIRUS

The identity of the plum virus, with respect to the virus diseases of other crops, is not known and there is at present no basis for assuming that the plum virus may affect other plants than those to which it has been transferred. However, it is of the same general nature as the ringspot virus of tobacco and the delphinium virus, so far as symptoms are concerned, and as both of these have a wide host range, it is possible that the plum virus may not be limited to plums and peaches. The aphid, *Myzus persicae*, is known to transmit certain viruses and as part of its life history is passed on peaches and plums this virus could

* In 1931 two Elberta trees from this nursery were inoculated by inserting "buds" from an affected Abundance plum. Soon after growth commenced in 1932, the typical patterns were found on a few of the Elberta leaves, but shortly afterward patterns were difficult to find. It will, therefore, probably be difficult to recognize this disease, if it occurs in the Elberta variety, by leaf symptoms.

have been transmitted to these trees from some other genera of plants. Disregarding the source of the virus, trees should not be propagated for sale from trees known to harbor the virus, but bud sticks should be obtained from old, vigorous trees which, from their past history, are known to be in a satisfactory state of health. Because of the uncertainty of diagnosis of the disease in peach trees, it appears to be a desirable precaution for the nurseryman in whose propagating plot the disease has been found, to obtain young trees, of the several varieties which he is propagating, from elsewhere and use the wood from them to replace his own budding stock. This could be done at very little cost and would be an added protection to the fruit grower who is depending upon the nurseryman and the constituted authorities to protect him in matters of this kind.